Exhibit 2

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CHAPTER TWELVE

CYTOKINES

In Chapter 1, we introduced the concept that defense against foreign organisms, such as viruses or bacteria, is mediated by natural (or innate) and by specific (or acquired) immunity. The effector phases of both natural and specific immunity are in large part mediated by protein hormones called **cytokines.** In natural immunity, the effector cytokines are mostly produced by mononuclear phagocytes and are therefore often called **monokines.** Although monokines can be elicited directly by microbes, they also can be secreted by mononuclear phagocytes in response to antigen-stimulated T cells, i.e., as part of specific immunity. Most cytokines in specific immunity are produced by activated T lymphocytes, and such molecules are commonly called **lymphokines.** T cells produce several cytokines that serve primarily to regulate the growth and differentiation of various lymphocyte populations and thus play important roles in the (**activation phase**) of T cell–dependent immune responses. Other T-cell–derived cytokines function principally to activate and regulate inflammatory cells, such as mononuclear phagocytes, neutrophils, and eosinophils. These T cell–derived cytokines are the effector molecules of cell-mediated immunity and are also responsible for communication between the cells of the immune and inflammatory systems. Finally, both lymphocytes and mononuclear phagocytes produce other cytokines, such as **colony-stimulating factors** (CSFs), which stimulate the growth and differentiation of immature leukocytes in the bone marrow, providing a source of additional leukocytes to replace the cells that are consumed during inflammatory reactions.

In this chapter, we discuss the structure, production, and biologic actions of cytokines. Before describing the specific molecules, we will begin with a brief historical overview of cytokine research and a review of the general properties shared by cytokines that allow us to consider them as a group.

DISCOVERY AND CHARACTERIZATION OF CYTOKINES

The discovery of particular cytokines can often be traced to investigation of infectious disease or of antigen-induced immune responses. Early studies of cytokines, extending from about 1950 to 1970, largely involved the description of numerous protein factors produced by different cells that mediated particular functions in particular bioassays. It was in this era, for example, that antiviral interferons, fever-producing pyrogens, and macrophage-activating factor were discovered. The second phase of cytokine research, encompassing roughly the 1970s, involved the partial purification and characterization of many individual cytokines as well as production of specific neutralizing antisera. In this period, it was first appreciated that diverse cytokine-mediated effects being studied by different investigators were often mediated by the same molecules. For example, interferon-γ (IFN-γ) was discovered by virologists as a T cell–derived antiviral protein and independently discovered by immunologists as a T cell–derived activator of macrophage functions. Similarly, interleukin-1 (IL-1) was discovered as an endogenous mediator of fever (a pyrogen) produced in response to bacterial infections and was discovered by immunologists as a costimulator of thymocyte proliferation. An important hypothesis generated at this time was that cytokines were principally synthesized by leukocytes and primarily acted on (other) leukocytes, and thus could be called **interleukins** (ILs). For example, a macrophage-derived costimulator activity for thymocytes was designated interleukin-1, and a T cell–derived T cell growth factor was called interleukin-2. However, preparations of cytokines available in the 1970s were often impure, and many of the available anticytokine antibodies were not absolutely specific for one cytokine. These methodological limitations prevented firm identification of the active factors as the same or distinct molecules.

The golden age of cytokine research occupied the 1980s. It was characterized by the molecular cloning and expression of individual cytokine molecules and by the production of completely specific, often monoclonal, neutralizing antibodies. These reagents allowed definitive identification of the structure and properties of individual cytokine molecules. The 1980s were more than a culmination of the early work because, in addition, many new cytokines were discovered and many previously unexpected properties of known cytokines were revealed. As a result of these studies, there is now a wealth of information about the sources and biologic activities of particular cytokines.

There are two continuing challenges in cytokine research. First, although much has been learned about the effects of cytokines *in vitro,* it is still largely unknown which biologic actions of a particular cytokine are important *in vivo* and which effects are necessary for a particular biologic response to occur. Experiments designed to answer these questions are in progress using recombinant cytokine molecules, specific cytokine antagonists, transgenic animals expressing cytokine genes, and animals that lack specific cytokines through gene knockout technology. Second, the availability of recombinant cytokines and specific antagonists has opened the possibility for clinicians to modify immune and inflammatory responses in a predictable fashion to influence the course of a disease. The task is to discover the most efficacious ways to use these **biological response modifiers** to achieve a desired outcome. Some of these clinical uses of cytokines and their antagonists will be discussed in subsequent chapters on immunologic diseases, transplantation, and tumor immunity.

GENERAL PROPERTIES OF CYTOKINES

Although cytokines are a diverse group of proteins, there are a number of properties shared by these molecules:

1. *Cytokines are produced during the effector phases of natural and specific immunity and serve to mediate and regulate immune and inflammatory responses.* In natural immunity, microbial products, such as lipopolysaccharide (LPS), directly stimulate mononuclear phagocytes to secrete their cytokines. In contrast, T cell–derived cytokines are elicited primarily in response to specific recognition of foreign antigens. However, these distinctions are not absolute because cytokines produced by one cell type often regulate the synthesis of cytokines by other cells.

2. *Cytokine secretion is a brief, self-limited event.* In general, cytokines are not stored as pre-formed molecules, and their synthesis is initiated by new gene transcription. Such transcriptional activation is usually transient, and the mRNAs encoding cytokines are unstable. The combination of a short period of transcription and a short-lived mRNA transcript ensures that cytokine synthesis is transient. Some cytokines may be additionally controlled by post-transcriptional mechanisms, such as proteolytic release of an active product from an inactive precursor. Once synthesized, cytokines are usually rapidly secreted, resulting in a burst of cytokine release as needed.

3. *Many individual cytokines are produced by multiple diverse cell types.* To emphasize that the cellular source of these molecules is usually not a distinguishing characteristic, investigators are increasingly adopting the convention followed in this book, namely to refer to these molecules collectively as cytokines rather than as lymphokines or monokines, regardless of their cellular source in a particular experiment.

4. *Cytokines act upon many different cell types.* This property is called **pleiotropism.** The earlier view that cytokines are primarily molecules produced by leukocytes that act particularly on leukocytes ("interleukins") is now considered too restricted a concept.

5. *Cytokines often have multiple different effects on the same target cell.* Some effects may occur simultaneously, whereas others may occur over different time frames (i.e., minutes, hours, or days).

6. *Cytokine actions are often redundant.* Many functions originally attributed to one cytokine have proved to be shared properties of several different cytokines. This observation has been reinforced by the study of knockout mice that lack particular cytokine genes yet display only subtle abnormalities in their immune responses.

7. *Cytokines often influence the synthesis of other cytokines,* leading to cascades in which a second or third cytokine may mediate the biologic effects of the first cytokine. The ability of one cytokine to enhance or suppress the production of others may provide important positive and negative regulatory mechanisms for immune and inflammatory responses.

8. *Cytokines often influence the action of other cytokines.* Two cytokines may interact to antagonize each other's action, to produce additive effects, or, in some cases, to produce greater than anticipated or even unique effects, a kind of interaction commonly referred to as synergy.

9. *Cytokines, like other polypeptide hormones, initiate their action by binding to specific receptors on the surface of target cell* (Box 12–1). The relevant target cell may be the same cell that secretes the cytokine (**autocrine** action), a nearby cell (**paracrine** action), or, like true hormones, a distant cell that is stimulated via cytokines that have been secreted into the circulation (**endocrine** action). Receptors for cytokines often show very high affinities for their ligands, with dissociation constants (K_d) in the range of 10^{-10} to 10^{-12} M. (For comparison, recall that antibodies typically bind antigens with a K_d of 10^{-7} M to 10^{-11} M, and major histocompatibility complex [MHC] molecules bind peptides with a K_d of only about 10^{-6} M.) As a consequence, only very small quantities of a cytokine need be produced to elicit a biologic effect.

10. *The expression of many cytokine receptors is regulated by specific signals.* This signal may be another cytokine or even the same cytokine that binds to the receptor, permitting positive amplification or negative feedback.

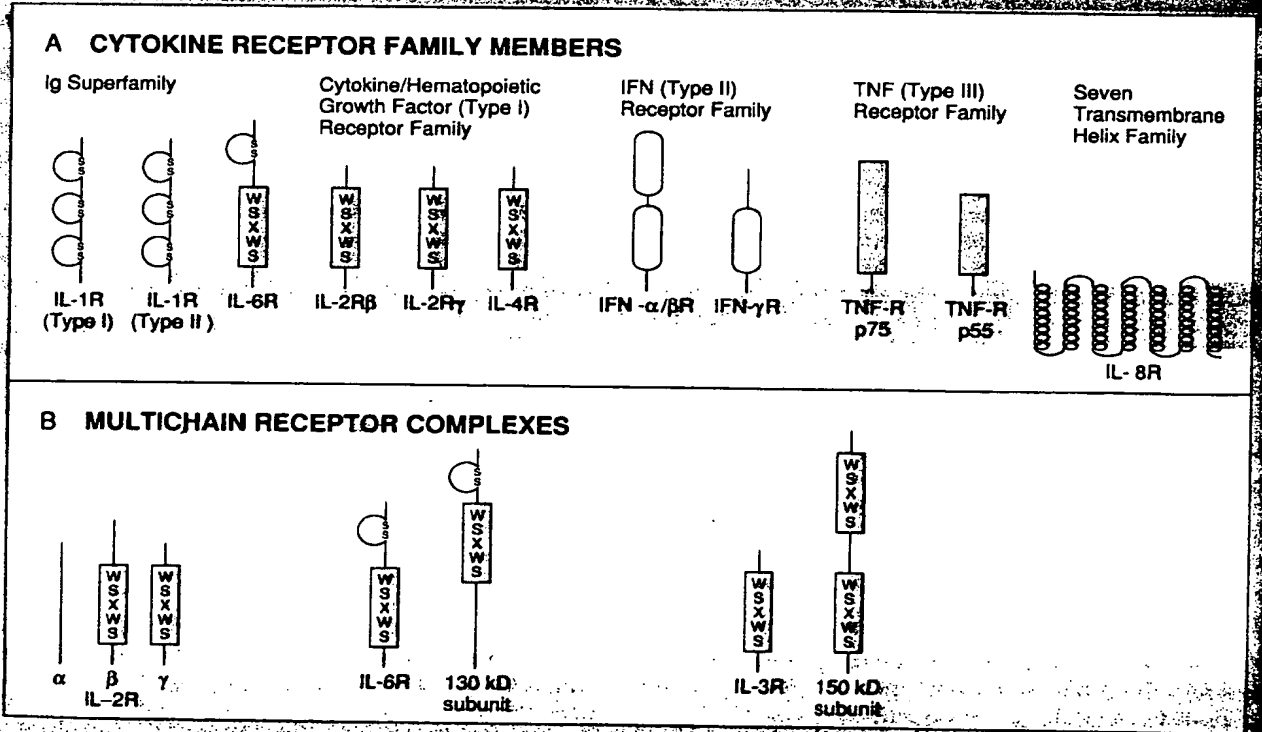
11. *Most cellular responses to cytokines require new mRNA and protein synthesis.* The mechanism by which cytokine binding to cell surface receptors stimulates transcription is still not completely known (Box 12–2). Some recent studies have identified nucleotide sequences in the 5′ flanking regions of genes whose transcription is activated by cytokine action. It is presumed that cytokines stimulate the production or binding of specific nuclear regulatory factors to these target sequences, and such binding, in turn, causes transcription.

12. *For many target cells, cytokines act as regulators of cell division, i.e., as growth factors.* Some immunologists now feel that cytokines should be categorized with epithelial and mesenchymal cell growth factors into a larger functional group of polypeptide regulatory molecules. However, we will continue to distinguish those molecules whose primary actions are as mediators of host defense (i.e., cytokines) from those molecules whose primary role resides in tissue repair (i.e., the epithelial and mesenchymal cell polypeptide growth factors).

FUNCTIONS OF CYTOKINES

We have organized our discussion of specific cytokines into four broad categories of function: (1) *mediators of natural immunity,* which are elicited by infectious agents from mononuclear phagocytes; (2) *regulators of lymphocyte activation, growth, and differentiation,* which are elicited in response to specific antigen recognition by T lymphocytes; (3) *regulators of immune-mediated inflammation,* which activate nonspecific inflammatory cells elicited in response to specific antigen recognition by T lymphocytes; and (4) *stimulators of immature leukocyte growth and differentiation,* which are produced by both stimulated lymphocytes and other cells. This classification is based on what appear to be the principal biologic actions of a

BOX 12–1. CYTOKINE RECEPTORS



Cytokine receptors share various structural motifs, allowing them to be categorized into families (A). Several of these receptor proteins are known to function as parts of multichain complexes (B). The 130 kD subunit that transduces IL-6 signals also transduces signals for other polypeptide receptors; whereas the 150 kD subunit that transduces IL-3 signals is shared by the IL-5 and GM-CSF receptors.

Continued

motif was originally found in β-adrenergic receptors and retinal rhodopsin and is widely shared by receptors that are coupled to heterotrimeric GTP-binding proteins.

Several cytokine receptors actually consist of two or more separate transmembrane polypeptide chains that function as a complex (see figure, part B). For example, the high affinity IL-2 receptor contains three separate chains (α, β, and γ). Both β and γ, but not α, contain the WSXWS motif. IL-2R$\beta\gamma$ heterodimers bind IL-2 and mediate signal transduction. IL-2Rα serves to increase the affinity of the receptor for the cytokine, but does not contribute to signaling. The γ subunit may also associate with the IL-4 and IL-7 receptors. The IL-6 receptor interacts with a separate transmembrane signal-transducing 130 kD glycoprotein that, like the IL-6 receptor itself, also contains an Ig domain and a WSXWS motif. At least three other protein hormones (ciliary neurotropic factor, oncostatin M, and leukemia inhibitory factor) also have receptors that interact with the same 130 kD signal-transducing molecule. Several other cytokine receptors (for IL-3, IL-5, and GM-CSF) are believed to share a 150 kD signal-transducing subunit in humans; in mice, the IL-3 receptor has a unique but homologous signal-transducing subunit.

Except for the M-CSF receptor and c-kit, which are tyrosine kinases, the molecular features of cytokine receptors that are involved in signal transduction are not well defined. This may change in the near future as many laboratories are currently working on this question.

particular cytokine, although, as we shall see, many cytokines may function in more than one of these categories.

Cytokines That Mediate Natural Immunity

The cytokines that mediate natural immunity include those that protect against viral infection and those that initiate inflammatory reactions that protect against bacteria. The cytokines discussed here are summarized in Table 12–1.

TYPE I INTERFERON

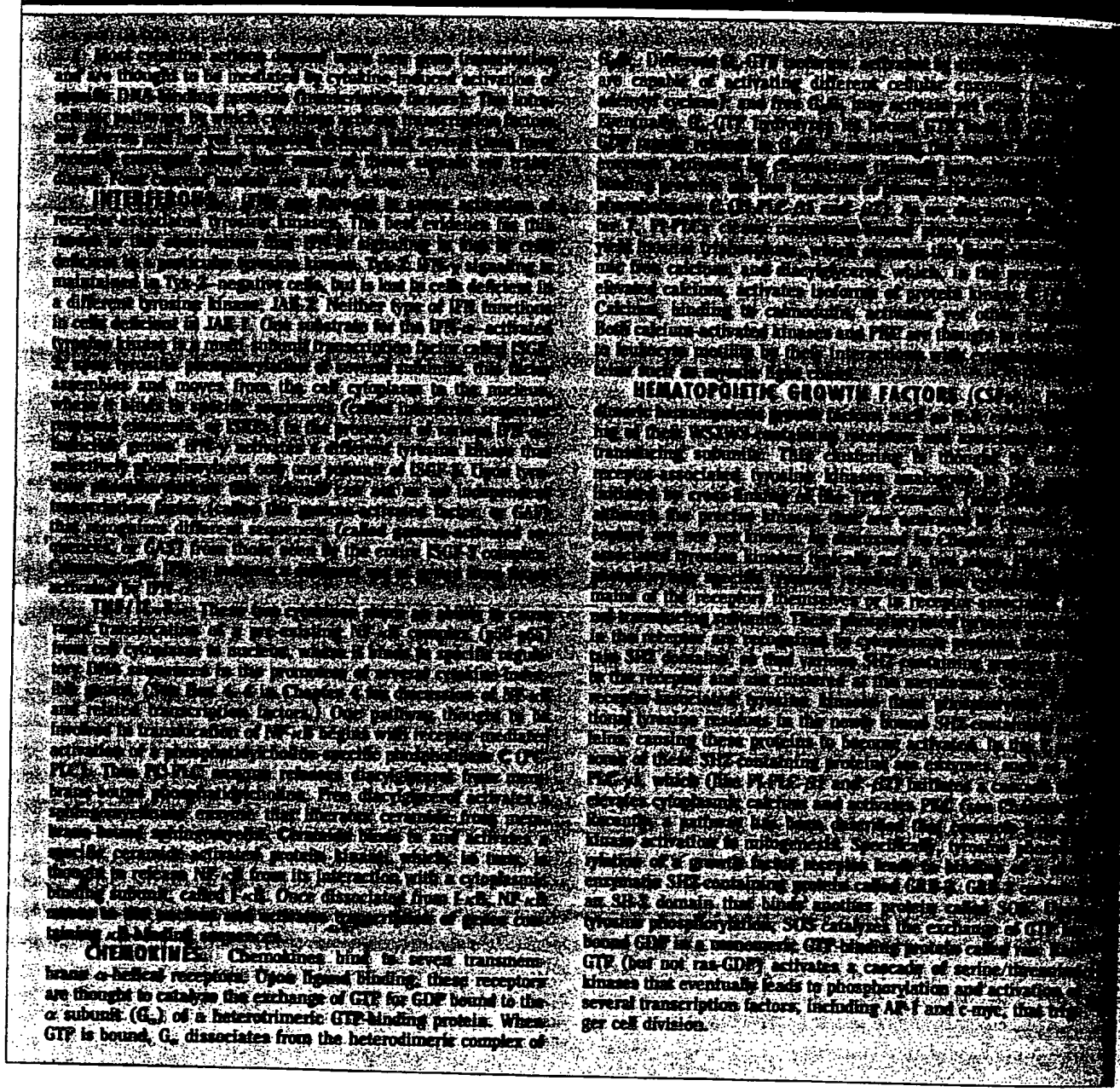
Type I interferons (IFNs) comprise two serologically distinct groups of proteins. The first group, collectively called IFN-α, is a family of about 20 structurally related polypeptides of approximately 18 kD, each encoded by a separate gene. (Some investigators now subdivide the IFN-α family into two groups, IFN-α1 and IFN-α2/IFN-ω, on the basis of the relatedness of amino acid sequences within the family.) Natural IFN-α preparations are usually a mixture of these molecules, and neutralizing sera react with all members of the IFN-α family. The major cell source for production of IFN-α is

TABLE 12–1. Mediators of Natural Immunity

Cytokine	Number of Genes	Polypeptide Size	Cell Source	Cell Target	Primary Effects on Each Target
Type I IFN	~20 IFN-α; 1 IFN-β	18 kD (monomer)	Mononuclear phagocyte, other (α); fibroblast, other (β)	All	Antiviral, antiproliferative, increased class I MHC expression
				NK cell	Activation
Tumor necrosis factor	1	17 kD (homotrimer)	Mononuclear phagocyte, T cell	Neutrophil	Activation (inflammation)
				Endothelial cell	Activation (inflammation, coagulation)
				Hypothalamus	Fever
				Liver	Acute phase reactants (serum amyloid A protein)
				Muscle, fat	Catabolism (cachexia)
				Thymocyte	Costimulator
Interleukin-1	2 (IL-1α, IL-1β)	17 kD (monomer)	Mononuclear phagocyte, other	Thymocyte	Costimulator
				Endothelial cell	Activation (inflammation, coagulation)
				Hypothalamus	Fever
				Liver	Acute phase reactants (serum amyloid A protein)
				Muscle, fat	Catabolism (cachexia)
Interleukin-6	1	26 kD (homodimer)	Mononuclear phagocyte, endothelial cell, T cell	Thymocyte	Costimulator
				Mature B cell	Growth
				Liver	Acute phase reactants (fibrinogen)
Chemokines	20+ related genes	8–10 kD (monomer)	Mononuclear phagocyte, endothelial cell; fibroblast; T cell; platelet	Leukocytes	Leukocyte chemotaxis and activation

Abbreviations: MHC, major histocompatibility complex; NK, natural killer; kD, kilodalton; IFN, interferon; IL, interleukin.

BOX 12–2. CYTOKINE SIGNAL TRANSDUCTION



the mononuclear phagocyte, and IFN-α is sometimes called **leukocyte interferon.** The second serological group of type I IFN consists of a single gene product, a 20 kD glycoprotein called IFN-β. The usual cell source for isolation of IFN-β is the cultured fibroblast, and IFN-β is sometimes called **fibroblast interferon.** However, many cells make both IFN-α and IFN-β. The most potent natural signal that elicits type I IFN synthesis is viral infection. Experimentally, production of type I IFN is commonly elicited by synthetic double-stranded RNA molecules, which may mimic a signal produced during viral replication. Both IFN-α and IFN-β are also secreted during immune responses to antigens. In this case, antigen-activated T cells stimulate mononuclear phagocytes to synthesize IFN. IFN-α and IFN-β show little structural similarity to each other. Nevertheless, all type I IFN molecules bind to the same cell surface receptor and appear to induce a similar series of cellular responses. The type I IFN receptor is a single chain polypeptide, homologous to the type II (immune or

gamma) IFN receptor. It may also share folding motifs with other proteins, such as tissue factor (see Box 12–1).

There are four principal biologic actions of type I IFN:

1. *Type I IFN inhibits viral replication.* IFN causes cells to synthesize a number of enzymes, such as 2′-5′ oligoadenylate synthetase, that collectively interfere with replication of viral RNA or DNA. The antiviral action of type I IFN is primarily paracrine, in that a virally infected cell secretes IFN to protect neighboring cells not yet infected. A cell that has responded to IFN and is resistant to viral infection is said to be in an **antiviral state.**

2. *Type I IFN inhibits cell proliferation.* This may be due to induction of the same enzymes that inhibit viral replication but also may involve other enzymes that prevent amino acid synthesis, especially of essential amino acids such as tryptophan. Although the mechanisms may be partly different, the antiviral effects and the antiproliferative effects of IFN cannot be uncoupled. It has been proposed that IFN-β is a physiologic inhibitor of normal cell growth. IFN-α is used as an antiproliferative agent for certain tumors (e.g., hairy cell leukemia and childhood hemangiomas).

3. *Type I IFN increases the lytic potential of natural killer (NK) cells.* As will be discussed in Chapter 13, a major function of NK cells is to kill virally infected cells.

4. *Type I IFN modulates MHC molecule expression.* In general, type I IFN increases expression of class I MHC molecules and profoundly inhibits class II MHC molecule expression. Because most cytolytic T lymphocytes (CTLs) recognize foreign antigens bound to class I MHC molecules, type I IFN boosts the effector phase of cell-mediated immune responses by enhancing the efficiency of CTL-mediated killing. At the same time, type I IFN may inhibit the cognitive phase of immune responses by preventing the activation of class II MHC–restricted helper T lymphocytes.

Thus, three of the principal activities of type I IFN, namely the induction of the antiviral state, the activation of NK cell lytic functions, and the increase in class I MHC molecule expression on virally infected cells, all act in concert to eradicate viral infections.

TUMOR NECROSIS FACTOR

Tumor necrosis factor (TNF) *is the principal mediator of the host response to gram-negative bacteria* and may also play a role in the response to other infectious organisms. (Some investigators refer to TNF as TNF-α and refer to lymphotoxin [LT] as TNF-β; this practice is controversial and increasingly confusing since the introduction of the term "LT-β" to refer to yet another member of this cytokine family. We shall use the simpler nomenclature of TNF and LT throughout this book.) The active components of gram-negative bacteria are **lipopolysaccharide** (LPS) molecules (also called **endotoxin**) derived from the bacterial cell wall (Fig. 12–1). TNF was originally identified (and was so named) as a mediator of tumor necrosis present in the

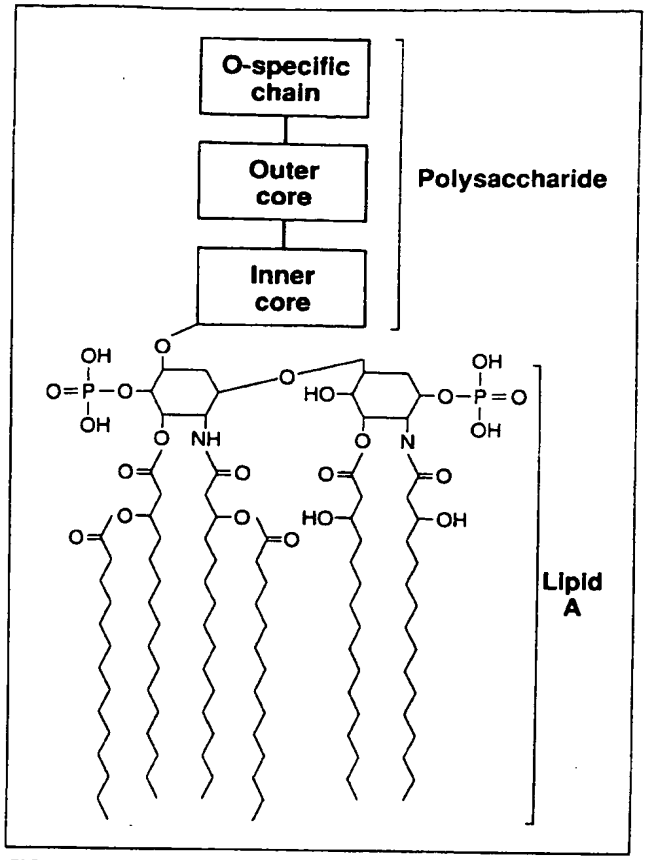


FIGURE 12–1. Structure of lipopolysaccharide. *Lipopolysaccharides are released when the cell walls of gram-negative bacteria, such as E. coli, are degraded. The lipid A moiety, which contains most of the biologic activity, is hydrophobic. The polysaccharide, which can contain 50 or more hexose moieties, can be divided into more conserved core regions and bacterial stain-specific ("O-specific") regions.*

serum of animals treated with LPS. At low concentrations, LPS stimulates the functions of mononuclear phagocytes and (in mice) acts as a polyclonal activator of B cells (see Chapters 9 and 13), host responses that contribute to elimination of the invading bacteria. However, high concentrations of LPS cause tissue injury, disseminated (widespread) intravascular coagulation (DIC), and shock, often resulting in death. The Shwartzman reaction is an experimental model for studying the pathologic effects of LPS (Box 12–3). It is now clear that TNF is one of the principal mediators of these effects of LPS.

The major cellular source of TNF is the LPS-activated mononuclear phagocyte, although antigen-stimulated T cells, activated NK cells, and activated mast cells can also secrete this protein. IFN-γ, produced by T cells, augments TNF synthesis by LPS-stimulated mononuclear phagocytes. Thus, TNF is a mediator of both natural and acquired immunity and an important link between specific immune responses and acute inflammation. In the mononuclear phagocyte, TNF is initially synthesized as a nonglycosylated transmembrane protein of approximately 25 kD. The orientation of

BOX 12–3. THE SHWARTZMAN REACTION

membrane TNF is unusual, in that the amino terminus is intracellular, the transmembrane segment is near the amino terminus, and the carboxy terminus is extracellular. A 17 kD fragment, including the carboxy terminus, is proteolytically cleaved off the plasma membrane of the mononuclear phagocyte to produce the "secreted" form, which circulates as a stable homotrimer of 51 kD. Native TNF assumes a triangular pyramidal shape such that each side of the pyramid is formed by a different monomeric subunit. The receptor binding sites are at the base of the pyramid, allowing simultaneous binding to more than one receptor.

TNF actions are initiated by binding of the soluble trimer to cell surface receptors. There are two distinct TNF receptors, of 55 and 75 kD, respectively, each encoded by a separate gene. The affinity of TNF for its receptors is unusually low for a cytokine, the K_d being only approximately 5×10^{-10} M for binding to the 75 kD receptor and 1×10^{-9} M for binding to the 55 kD receptor. However, TNF is synthesized in very large quantities and can easily saturate its receptors. TNF receptors are present on almost all cell types examined. Activated cells shed their TNF receptors; such soluble receptors may act as competitive inhibitors of the cell surface receptor.

Many TNF responses involve increased rates of transcription of particular target genes, often through activation of NF-κB or AP-1 transcription factors. A model for the activation of NF-κB by TNF is described in Box 12–2.

The biologic actions of TNF, like those of LPS, are best understood as a function of quantity (Fig. 12–2). *At low concentrations, i.e., at approximately 10^{-9} M, TNF acts locally as a paracrine and autocrine regulator of leukocytes and endothelial cells.* The principal biologic actions of TNF at low concentrations are the following:

1. TNF causes vascular endothelial cells to express new surface receptors (**adhesion molecules**) that make the endothelial cell surface become adhesive for leukocytes, initially for neutrophils and subsequently for monocytes and lymphocytes. TNF also acts on neutrophils to increase their adhesiveness for endothelial cells. These actions contribute to accumulation of leukocytes at local sites of inflammation and are probably the physiologically most important local effects of TNF (see Chapter 13).

2. TNF activates inflammatory leukocytes to kill microbes. TNF is especially potent at activating neutrophils but also affects eosinophils and mononuclear phagocytes.

3. TNF stimulates mononuclear phagocytes and other cell types to produce cytokines, including IL-1, IL-6, TNF itself, and chemokines.

4. TNF exerts an interferon-like protective effect against viruses and augments expression of class I MHC molecules, potentiating CTL-mediated lysis of virally infected cells.

These effects of TNF are critical for inflammatory responses to microbes. If inadequate quantities of TNF are present, e.g., in animals treated with neutralizing anti-TNF antibodies, a consequence may be a failure to contain infections.

If the stimulus for TNF production is sufficiently strong, greater quantities of the cytokine are produced. In this setting, TNF enters the blood stream, where it can act as an endocrine hormone. *The principal systemic actions of TNF in physiologic host responses to infections are the following:*

1. TNF is an *endogenous pyrogen* that acts on cells in hypothalamic regulatory regions of the brain to induce fever. It shares this property with IL-1, and both cytokines are found in the serum of animals or people

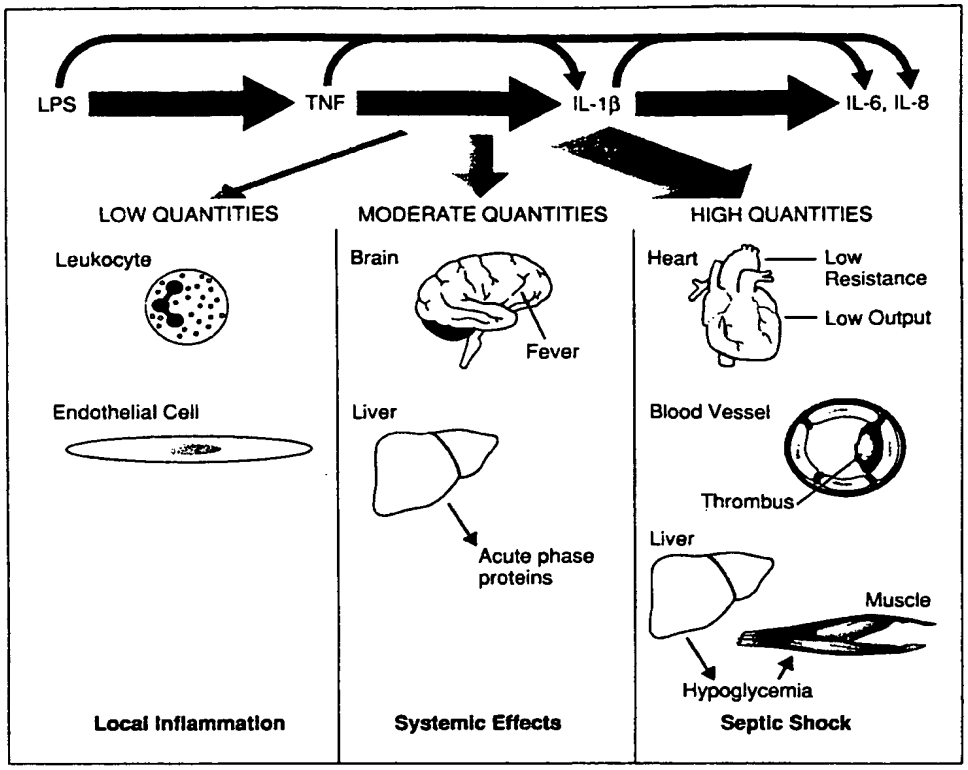


FIGURE 12–2. The LPS-induced cytokine cascade. *Bacterial LPS acts on macrophages to release TNF. TNF induces macrophages to release IL-1β. IL-1β acts on macrophages and vascular endothelial cells to release IL-6 and IL-8. (The thinner arrows indicate that LPS directly induces IL-1β, IL-6, and IL-8 and that TNF directly induces IL-6 and IL-8, but these actions are amplified through the cascade.) When low quantities of cytokine are released, the effects are local. With moderate quantities, systemic effects can be detected. At high levels, these cytokines produce the syndrome of septic shock.*

exposed to LPS, which functions as an exogenous pyrogen. Fever production in response to TNF or IL-1 is mediated by increased synthesis of prostaglandins by cytokine-stimulated hypothalamic cells. Prostaglandin synthesis inhibitors, such as aspirin, reduce fever by blocking this action of TNF or IL-1.

2. TNF acts on mononuclear phagocytes and perhaps vascular endothelial cells to stimulate secretion of IL-1 and IL-6 into the circulation (Fig. 12–3). This is one example of a cascade of cytokines that share many biologic activities.

3. TNF acts on hepatocytes to increase synthesis of certain serum proteins, such as serum amyloid A protein. The spectrum of hepatocyte proteins induced by TNF is identical to that induced by IL-1 but differs from that induced by IL-6 (described below). The com-

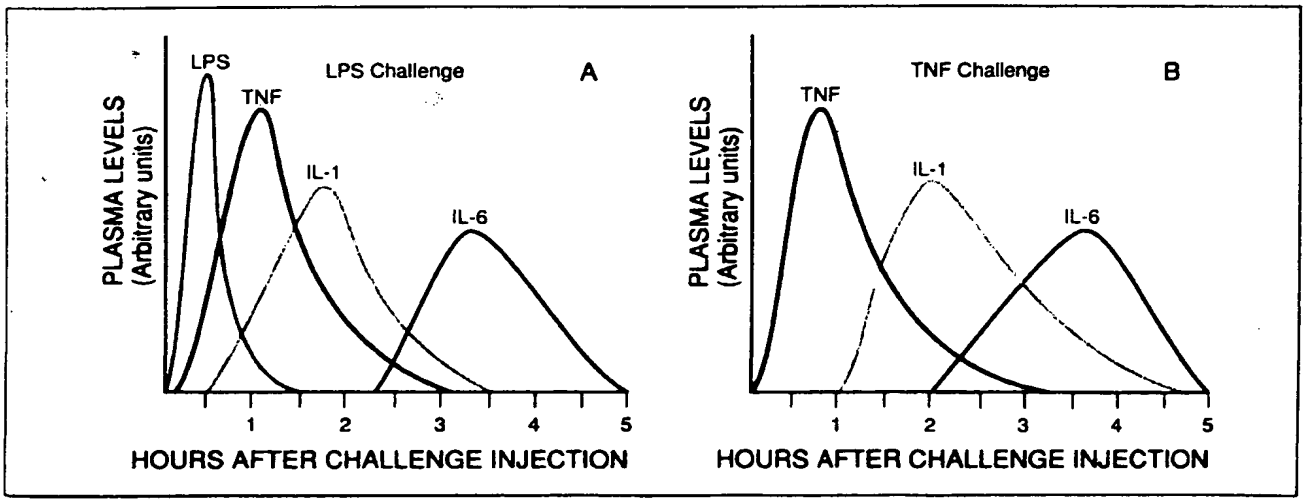


FIGURE 12–3. Cytokine cascades in sepsis. *Following injection of lipopolysaccharide (LPS) (A), there are successive waves of tumor necrosis factor (TNF), IL-1, and IL-6 detectable in plasma. Injection of TNF (B) produces successive waves of IL-1 and IL-6. In the presence of antibody to TNF, LPS-induced plasma elevations of IL-1 and IL-6 are inhibited; and in the presence of antibody to IL-1, plasma elevations of IL-6 are inhibited. These data suggest that there are ordered cascades of cytokine production: LPS induces TNF, which induces IL-1, which induces IL-6 synthesis.*

bination of hepatocyte-derived plasma proteins induced by TNF or IL-1 plus those induced by IL-6 constitutes the **acute phase response** to inflammatory stimuli (Box 12–4).

4. TNF activates the coagulation system, primarily by altering the balance of the procoagulant and anticoagulant activities of vascular endothelium.

5. TNF suppresses bone marrow stem cell division. Chronic administration of TNF may lead to lymphopenia and immunodeficiency.

6. Long-term systemic administration of TNF to experimental animals causes the metabolic alterations of **cachexia,** a state characterized by wasting of muscle and fat cells. The cachexia is produced largely by TNF-induced appetite suppression. TNF also suppresses synthesis of lipoprotein lipase, an enzyme needed to release fatty acids from circulating lipoproteins so that they can be utilized by the tissues. Although TNF by itself can produce cachexia in experimental animals, other cytokines, such as IL-1, may also contribute to the cachectic state accompanying certain chronic diseases such as tuberculosis and cancer.

The combination of fever, elevated IL-6 levels, elevated acute phase reactants, bone marrow suppression, and activation of coagulation has been noted in patients treated with intravenous TNF for cancer chemotherapy.

In the setting of gram-negative bacterial sepsis, massive quantities of TNF are produced, and serum concentrations of TNF can transiently exceed 10^{-7} M. Animals producing this much TNF die of circulatory collapse and disseminated intravascular coagulation. Neutralizing antibodies to TNF can prevent mortality, implicating this cytokine as a critical mediator of septic or endotoxin shock (Fig. 12–4). Moreover, infusion of high levels of TNF is by itself lethal, producing a shock-like syndrome. *Several specific actions of TNF may contribute to its lethal effects at extremely high concentrations.*

1. TNF reduces tissue perfusion by depressing myocardial contractility. The mechanism of this action appears to involve induction of an enzyme in cardiac myocytes, nitric oxide synthase (NOS), that converts arginine to citrulline and NO. NO made by this enzyme inhibits myocardial contractility.

2. TNF further reduces blood pressure and tissue perfusion by relaxing vascular smooth muscle tone. TNF may act directly on smooth muscle cells and also can act indirectly by stimulating production of vasodilators, such as prostacyclin and NO by vascular endothelial cells.

3. TNF causes intravascular thrombosis, leading to reduced tissue perfusion. This is due to a combination of endothelial and mononuclear phagocyte alterations, which promote coagulation, and activation of neutrophils leading to vascular plugging by these cells. These TNF-mediated actions account for many of the effects of LPS seen in the Shwartzman reaction of rabbits and disseminated intravascular coagulation in humans.

4. TNF causes severe metabolic disturbances,

BOX 12–4. THE ACUTE PHASE RESPONSE

The acute phase response consists of a rapid adjustment of plasma protein composition in response to injurious stimuli, including infection, burns, trauma, and neoplasia. Several different plasma proteins rise in concentration, whereas others fall. Among the proteins whose levels increase are C-reactive protein, which functions as a nonspecific opsonin to augment phagocytosis of bacteria; α₂-macroglobulin and other anti-proteinases, that clear circulating proteins; fibrinogen; and serum amyloid A protein, a molecule of uncertain function. Albumin and transferrin, the iron transport protein, decline. Most of these changes in plasma concentrations can be directly attributed to alterations in the levels of synthesis of these plasma proteins by hepatocytes. Experiments using whole animals, liver slices, cultured hepatocytes, or hepatocyte-derived tumor cell lines have revealed that these changes in biosynthesis are caused by alterations in gene transcription regulated primarily by IL-6 (on fibrinogen) and IL-1/TNF (on serum amyloid protein).

The precise function of the acute phase response is largely unknown. The increases in opsonizing proteins and anti-proteinases are believed to aid natural immunity and protect against tissue injury, respectively. Elevation in fibrinogen, caused by IL-6, is of uncertain benefit but has had major impact on clinical medicine. Specifically, elevated levels of fibrinogen can cause red blood cells to form stacks (rouleaux). When blood is collected and allowed to stand at unit gravity, rouleaux sediment more rapidly than individual red blood cells. Rouleaux in venous blood may sediment before the red blood cells are fully oxygenated, leading to a dark color in the tissues of the examining physician. [illegible] this sense of death, disorganized red blood cells were called "[illegible]," and ancient and medieval physicians would perform bleeding of patients to remove this "sickly humor." In modern times, this recognition that the more rapid red blood cell sedimentation reflected, not presence of illness, rather than representing a cause, allowed diagnosis of the erythrocyte sedimentation rate to become a useful diagnostic test for the presence of [illegible] phase response. In the past few years, more specific measures of the acute phase response, for example, of C-reactive protein or IL-6, have now largely supplanted this useful (but [illegible]

Although the acute phase response is characterized by rapid onset, it can persist in the setting of chronic inflammation. In some patients with chronic inflammatory disease (e.g., rheumatoid arthritis; see Chapter 20), persistent elevations of serum amyloid A protein may lead to deposition of this protein in the interstitium of tissues. Such deposited protein, in the form of fibrils rich in β-pleated sheet structure, can interfere with normal organ function (e.g., myocardial contraction, glomerular filtration). Such patients are said to have developed amyloidosis. The [illegible] cause such protein deposits stain with acidic iodine, a reaction originally developed for amylose or animal starch. Similar fibrils can develop in other settings (e.g., multiple myeloma, Alzheimer's disease, or endocrine cell tumors); however, in these cases, the protein fibrils are not of serum amyloid A protein origin and are unrelated to the acute phase response.

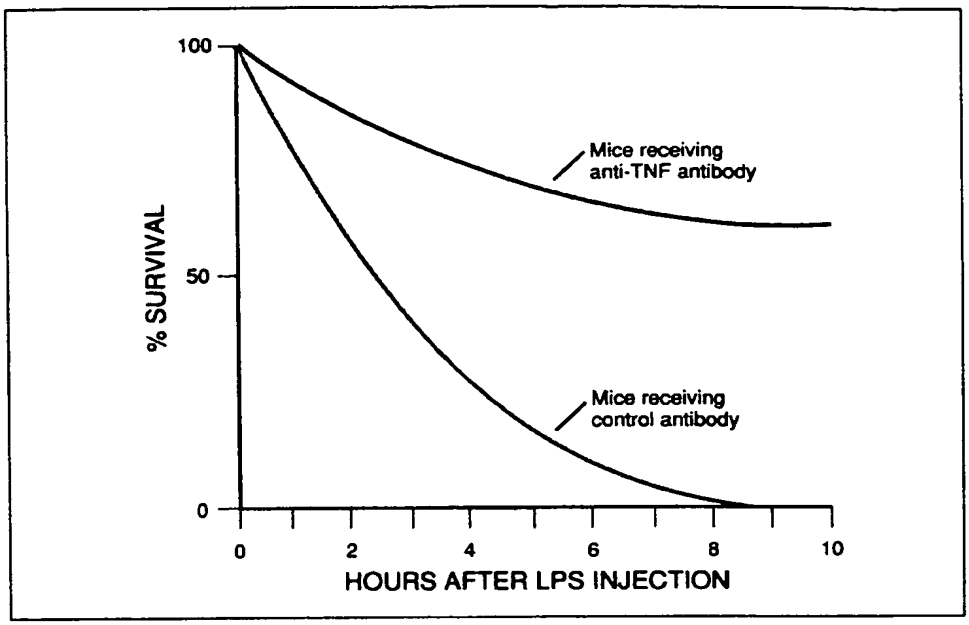


FIGURE 12–4. Tumor necrosis factor (TNF) mediates septic shock. *Antibody to TNF can significantly reduce mortality associated with gram-negative sepsis or injection of lethal concentrations of lipopolysaccharide (LPS).*

such as a fall in blood glucose concentrations to levels that are incompatible with life. This is due to overutilization of glucose by muscle and failure to replace glucose by the liver.

Many of the biologic actions of TNF are augmented by IFN-γ. In some cells that are targets of TNF effects, this interaction may be explained by IFN-γ–stimulated increases in TNF receptor numbers. However, in many cases, IFN-γ enhancement of TNF activity is noted without any effect on TNF binding. The full significance of this interaction is not clear, but activated T cells often secrete TNF and IFN-γ coordinately. Coordinate secretion of these two cytokines may provide a means of locally enhancing the actions of TNF without requiring concentrations that produce systemic toxicity.

INTERLEUKIN-1

Interleukin-1 was first defined as a polypeptide derived from mononuclear phagocytes that enhanced the responses of thymocytes to polyclonal activators, i.e., as a costimulator of T cell activation. A convenient bioassay for this activity is the costimulation (with concanavalin A or phytohemagglutinin) of murine thymocyte proliferation. It is now appreciated that this assay is not specific for IL-1 and may also detect other cytokines such as IL-6. Although IL-1 was discovered as a costimulator of T cells, *it is now clear that the principal function of IL-1, similar to TNF, is as a mediator of the host inflammatory response in natural immunity.* Indeed, there is little evidence to support a role of IL-1 as a physiologically important costimulator of mature T cell activation.

The major cellular source of IL-1, like that of TNF, is the activated mononuclear phagocyte. IL-1 production by mononuclear phagocytes can be triggered by bacterial products such as LPS, by macrophage-derived cytokines such as TNF or IL-1 itself, and by contact with $CD4^+$ T cells. Like TNF, IL-1 can be found in the circulation following gram-negative bacterial sepsis, where it can act as an endocrine hormone. In this case, it is produced mainly in response to TNF (see Fig. 12–3). IL-1 synthesis differs from that of TNF in two important regards. First, T cells are more effective than LPS at eliciting synthesis of IL-1 by mononuclear phagocytes. Second, IL-1 is made by many diverse cell types, such as epithelial and endothelial cells, providing potential local sources of IL-1 in the absence of macrophage-rich infiltrates.

Biochemical purification of IL-1 secreted by mononuclear phagocytes revealed that the biologic activity of this cytokine actually resided in two major polypeptide species, each approximately 17 kD but with distinct isoelectric points of 5.0 and 7.0. It is now known that these two forms, called IL-1α and IL-1β, respectively, are products of two different genes. The two forms of IL-1 show less than 30 per cent structural homology to each other, but both species bind to the same cell surface receptors and their biologic activities are essentially identical. A third member of the IL-1 family, called IL-1 receptor antagonist, will be discussed below. The IL-1 molecules are also related structurally to the various forms of fibroblast growth factor.

Both IL-1 polypeptides are synthesized as approximately 33 kD precursors that are proteolytically cleaved to generate the mature 17 kD proteins. The 17 kD forms fold in a barrel-like structure, rich in strands of β-pleated sheet. The 33 kD IL-1α precursor is biologically active, but IL-1β must be processed to the 17 kD form before it can exert biologic effects. An IL-1–specific protease has been identified in mononuclear phagoctyes that is responsible for most of the conversion of IL-1β to its active form. The complete amino acid

sequence of both IL-1 species presents a theoretical problem: unlike conventionally secreted proteins, neither IL-1 polypeptide has a hydrophobic signal sequence to target the nascent polypeptide to the endoplasmic reticulum, and both proteins appear to be synthesized as cytoplasmic proteins. It is therefore unknown how these molecules are secreted. The N terminal region of IL-1α contains a nuclear targeting sequence that may transport IL-1α into the nucleus of the cell that synthesizes it. Most of the IL-1 activity found in the circulation is IL-1β.

Two different membrane receptors for IL-1 have been characterized, both of which are members of the Ig superfamily. The type I receptor was initially characterized from a T cell line where it mediates IL-1 stimulation; it has slightly higher affinity for IL-1β than for IL-1α. The type II receptor was initially characterized from a B cell; it has greater affinity for IL-1α than for IL-1β. It is not clear, at present, whether the type II receptor mediates IL-1 actions or merely serves to competitively inhibit IL-1 binding to the type I receptor. The K_d for IL-1 binding to its receptors may be as high as 1×10^{-12} M; however, IL-1 may be active on some target cells at concentrations as low as 1×10^{-15} M, suggesting that additional IL-1 binding proteins may exist. Many IL-1–induced transcriptional effects, like those of TNF, involve NF-κB (see Box 12–2).

The biologic effects of IL-1, similar to those of TNF, depend on the quantity of cytokine released (see Fig. 12–2). At low IL-1 concentrations, the principal biologic effects are as a mediator of local inflammation. Specifically, IL-1 acts on mononuclear phagocytes and vascular endothelium to increase further synthesis of IL-1 and induce synthesis of IL-6. It also shares many of the inflammatory properties of TNF. For example, IL-1 acts on endothelial cells to promote coagulation and to increase expression of surface molecules that mediate leukocyte adhesion. IL-1 does not directly activate inflammatory leukocytes, such as neutrophils, but it causes mononuclear phagocytes and endothelial cells to synthesize chemokines that do activate leukocytes (see below).

When secreted in larger quantities, IL-1 enters the blood stream and exerts endocrine effects. *Systemic IL-1 shares with TNF the ability to cause fever, to induce synthesis of acute phase plasma proteins* (such as serum amyloid A protein) *by the liver, and to initiate metabolic wasting (cachexia).*

It was initially very surprising to note the extensive similarities of IL-1 actions with those of TNF, a striking example of the redundancy of cytokine effects. However, there are several important differences between these cytokines. First, IL-1 does not produce tissue injury by itself, although it is secreted in response to LPS and can potentiate tissue injury caused by TNF. Moreover, even at very high systemic concentrations, IL-1 is not lethal. Second, although IL-1 mimics many of the inflammatory and procoagulant properties of TNF, IL-1 cannot replace TNF as a mediator of the Shwartzman reaction and does not cause hemorrhagic necrosis of tumors. Third, most tumor cell lines are not directly lysed by IL-1 *in vitro*. Fourth, IL-1 does not share with TNF an ability to increase expression of MHC molecules. Finally, IL-1 potentiates rather than suppresses the actions of CSFs on bone marrow cells.

IL-1 is the only cytokine to date for which **naturally occurring inhibitors** have been described. The best defined of these is produced by human mononuclear phagocytes. It is structurally homologous to IL-1 and binds to IL-1 receptors but is biologically inactive, so that it functions as a competitive inhibitor of IL-1. It is therefore commonly called IL-1 receptor antagonist (IL-1ra). In monocytes, IL-1ra is synthesized with a signal sequence and is efficiently secreted, thereby inhibiting the actions of IL-1. In other cell types, IL-1ra mRNA may be spliced to remove the signal sequence so it is not secreted; the functions of intracellular IL-1ra are unknown. Type I and type II IL-1 receptors are also shed by activated cells. Both IL-1ra and soluble receptors may be endogenous regulators of IL-1 action. It may also be possible to use cytokine inhibitors as biologic response modifiers in disease states that are caused by excessive or unregulated cytokine production, such as septic shock.

INTERLEUKIN-6

Interleukin-6 (IL-6) is a cytokine of approximately 26 kD that is synthesized by mononuclear phagocytes, vascular endothelial cells, fibroblasts, and other cells in response to IL-1 and, to a lesser extent, TNF. It is also made by some activated T cells. IL-6 can be detected in the circulation following gram-negative bacterial infection or TNF infusion and appears to be secreted in response to TNF or IL-1 rather than LPS itself (see Fig. 12–3). IL-6 does not cause vascular thrombosis or the tissue injury that is seen in response to LPS or TNF. The functional form of IL-6 is probably a homodimer.

The receptor for IL-6 consists of a 60 kD binding protein and 130 kD signal-transducing subunit. The binding protein contains both an Ig domain and a tryptophan-serine-X-tryptophan-serine (WSXWS, where X stands for a variable amino acid residue) motif characteristic of receptors that interact with cytokines sharing a four α-helical folding pattern (see Box 12–1). The signal transducing subunit also contains both an Ig domain and the WSXWS motif, but it is not specific for IL-6 and can interact with other polypeptides as well. Clustering of the 130 kD subunit by interactions with cytokine and specific binding protein is thought to trigger signaling. Shed IL-6 receptors can also bind IL-6 and signal through the 130 kD subunit.

The two best described actions of IL-6 are on hepatocytes and B cells:

1. *Interleukin-6 causes hepatocytes to synthesize several plasma proteins, such as fibrinogen, that contribute to the acute phase response* (Box 12–4).

2. *Interleukin-6 serves as a growth factor for activated B cells late in the sequence of B cell differentiation.* IL-6 similarly acts as a growth factor for many malignant plasma cells (plasmacytomas or myelomas) and many plasmacytoma cells that grow autonomously actually secrete IL-6 as an autocrine growth factor. Moreover, IL-6 can promote the growth of somatic cell

hybrids produced by fusing normal B cells with plasmacytoma cells, i.e., the "hybridomas" that produce monoclonal antibodies (Box 3–1, Chapter 3). Transgenic mice that over-express the IL-6 gene develop massive polyclonal proliferation of plasma cells.

In addition to these well described actions, *in vitro* experiments suggest that IL-6 may serve as a costimulator of T cells and of thymocytes. IL-6 also acts as a cofactor with other cytokines for the growth of early bone marrow hematopoietic stem cells. Finally, it should be noted that one of the first activities ascribed to IL-6, that of an interferon, has not been confirmed using recombinant preparations of IL-6, and the alternative name of IFN-β_2 for this cytokine has now been abandoned.

CHEMOKINES

A recent discovery in the cytokine field is the existence of a large family of structurally homologous cytokines, approximately 8 to 10 kD in size. These molecules share the ability to stimulate leukocyte movement (**chemokinesis**) and directed movement (**chemotaxis**) and have been collectively called "chemokines," a contraction of **chemo**tactic cyto**kines.** All of these molecules contain two internal disulfide loops. Some investigators separate these factors into two subfamilies, based on whether the two amino terminal cysteine residues are immediately adjacent (cys-cys) or separated by one amino acid (cys-X-cys). These differences correlate with organization of the two subfamilies into separate gene clusters.

The chemokines of the cys-X-cys subfamily are produced largely by activated mononuclear phagocytes as well as by tissue cells (endothelium, fibroblasts) and megakaryocytes (which give rise to platelets containing stored chemokine). These molecules act predominantly on neutrophils as mediators of acute inflammation. The best characterized member of this subfamily is interleukin-8. The cys-cys subfamily is produced largely by activated T cells. These molecules act predominantly on subsets of mononuclear inflammatory cells. For example, a chemokine called RANTES acts on memory $CD4^+$ T cells and monocytes. An exception to this generalization is monocyte chemotactic protein–1 (MCP-1), a cys-cys chemokine that acts only on monocytes but is made by activated mononuclear phagocytes and tissue cells as well as by T cells. Chemokines of both subfamilies bind to heparan sulfate proteoglycans on the endothelial cell surface, and may function principally to stimulate chemokinesis of leukocytes that attach to cytokine-activated endothelium through induced adhesion molecules.

Several chemokine receptors have recently been characterized, and all of these belong to the seven transmembrane α-helical family (see Box 12–1). Interestingly, some chemokine receptors appear to interact with several different chemokines; the significance of this molecular promiscuity is not yet known.

Cytokines That Regulate Lymphocyte Activation, Growth, and Differentiation

Some cytokines function principally to regulate the growth and differentiation of lymphocytes and mediate the activation phase of specific immune responses. Most of these cytokines are produced by T cells, especially antigen-specific $CD4^+$ T lymphocytes. Such T cells provide help for both cell-mediated and humoral immune response, in large part through the secretion of cytokines. The cytokines that act primarily to regulate lymphocytes themselves are interleukin-2, interleukin-4, and transforming growth factor–β (TGF-β). The properties of the cytokines discussed in this section are listed in Table 12–2.

INTERLEUKIN-2

Interleukin-2 (IL-2), originally called ***T cell growth factor*** *(TCGF), is the principal cytokine responsible for progression of T lymphocytes from the G_1 to S phase of the cell cycle.* IL-2 is produced by $CD4^+$ T cells, and in lesser quantities by $CD8^+$ T cells. IL-2 acts on the same cells that produce it; i.e., it functions as an **autocrine**

TABLE 12–2. Mediators of Lymphocyte Activation, Growth, and Differentiation

Cytokine	Number of Genes	Polypeptide Size	Cell Source	Cell Target	Primary Effects on Each Target
Interleukin-2	1	14–17 kD (monomer)	T cells	T cell	Growth; cytokine production
				NK cell	Growth, activation
				B cell	Growth, antibody synthesis
Interleukin-4	1	20 kD (monomer)	$CD4^+$ T cell, mast cell	B cell	Isotype switching to IgE
				Mononuclear phagocyte	Inhibit activation
				T cell	Growth
Transforming growth factor–β	Several	14 kD (homodimer)	T cells, mononuclear phagocyte, other	T cell	Inhibit activation and proliferation
				Mononuclear phagocyte	Inhibit activation
				Other cell types	Growth regulation

Abbreviations: NK, natural killer; kD, kilodalton; Ig, immunoglobulin.

growth factor. IL-2 also acts on nearby T lymphocytes, including both $CD4^+$ and $CD8^+$ cells, and is also therefore a **paracrine growth factor.** During physiologic immune responses, IL-2 does not circulate in the blood to act at a distance, and thus it is not considered to be an endocrine growth factor.

Secreted IL-2 is a 14 to 17 kD glycoprotein encoded by a single gene on chromosome 4 in humans. The size heterogeneity of the mature protein is due to variable extents of glycosylation of an approximately 130 amino acid residue polypeptide. Native IL-2 is folded into a globular protein containing two sets of paired parallel α-helices, each sheet oriented at a slight angle to the other. This α-helical folding motif is common to all cytokines that interact with receptors with the WSXWS sequence, including IL-3, IL-4, IL-5, IL-6, GM-CSF, and G-CSF. Normally, IL-2 is transcribed, synthesized, and secreted by T cells only upon activation by antigens. IL-2 synthesis is usually transient, with an early peak of secretion occurring about 4 hours after activation. The mechanisms of transcriptional regulation of IL-2 synthesis have been described in Chapter 7 (Box 7–5).

The principal actions of IL-2 are on lymphocytes:

1. *Interleukin-2 is the major autocrine growth factor for T lymphocytes, and the quantity of IL-2 synthesized by activated $CD4^+$ T cells is an important determinant of the magnitude of T cell-dependent immune responses.* IL-2 also stimulates synthesis of other T cell–derived cytokines such as IFN-γ and lymphotoxin (LT). Failure to synthesize adequate quantities of IL-2 has been described as a cause of antigen-specific T cell anergy (see Chapter 10).

The action of IL-2 on T cells is mediated by binding to IL-2 receptor proteins. This system is perhaps the best understood of all cytokine receptors. Two distinct cell surface proteins on T cells bind IL-2. The first to be identified, called IL-2Rα, is a 55 kD polypeptide (p55) that appears upon T cell activation and was originally called Tac (for T activation) antigen. IL-2Rα binds IL-2 with a K_d of approximately 10^{-8} M. Binding of IL-2 to cells expressing only IL-2Rα does not lead to any detectable biologic response. The second IL-2–binding protein, called IL-2Rβ, is about 70 to 75 kD (called variously p70 or p75) and is a member of the receptor family characterized by the WSXWS motif (see Box 12–1). The affinity of binding of IL-2 to this receptor is higher than to IL-2Rα, with a K_d of approximately 10^{-9} M. IL-2Rβ is expressed coordinately with a 64 kD polypeptide, called IL-2Rγ, which is also a member of the WSXWS family, forming a complex designated as IL-2R$\beta\gamma$. IL-2 causes growth of cells expressing only IL-2R$\beta\gamma$, with half maximal growth stimulation occurring at the same concentration of IL-2 that produces half maximal binding. Cells that express IL-2Rα as well as IL-2R$\beta\gamma$ can bind IL-2 much more tightly, with a K_d of approximately 10^{-11} M. Growth stimulation of such cells occurs at a similarly low IL-2 concentration. Both IL-2 binding and growth stimulation can be blocked by antibodies to either IL-2Rα or IL-2Rβ and most efficiently by a combination of antibodies to both receptor subunits. These observations have been interpreted to mean that *IL-2Rα forms a complex with IL-2R$\beta\gamma$, increasing the affinity of the IL-2R$\beta\gamma$ receptor for IL-2 and thereby allowing a growth signal to be delivered at significantly lower IL-2 concentrations.* It is believed that IL-2 first binds rapidly to IL-2Rα, and this facilitates association with IL-2R$\beta\gamma$. As depicted in Figure 12–5, resting T cells express IL-2R$\beta\gamma$ but not IL-2Rα and can be stimulated only by high levels of IL-2. Upon antigen receptor-mediated T cell activation, IL-2Rα is rapidly expressed, thereby reducing the concentration of IL-2 needed for growth stimulation. In fact, IL-2 itself can further increase IL-2Rα synthesis. Although much is known about the interaction of IL-2 with its receptor, the intracellular signals produced by cytokine binding have not been identified. There is some evidence that a tyrosine kinase may be involved in IL-2-mediated T cell growth stimulation, but its identity is not yet known.

2. *IL-2 stimulates the growth of NK cells and enhances their cytolytic function,* producing so-called lymphokine-activated killer (LAK) cells (see Chapter 13). NK cells, like resting T cells, express IL-2R$\beta\gamma$ and can be stimulated by high levels of IL-2. NK cells, however, do not express IL-2Rα and therefore do not reduce their requirement for IL-2, even after activation. Thus, only high concentrations of IL-2 will lead to LAK cell formation. IL-2 synergizes with other cytokines, notably IL-12, to induce IFN-γ secretion by NK cells.

3. *IL-2 acts on human B cells both as a growth factor and as a stimulus for antibody synthesis.* It does not appear to cause isotype switching. These activities of IL-2 are discussed more fully in Chapter 9.

Actions of IL-2 on other cell populations are less well established. IL-2 receptor proteins have been detected on mononuclear phagocytes. However, a specific IL-2 function in this cell type has not been described. Mice in which the IL-2 gene is disrupted by knockout technology appear to have a relatively normal immune system. These observations suggest that many IL-2 actions may be redundant or, more likely, that IL-2–independent T cells can arise and be selected for in the thymus.

Chronic T cell stimulation leads to shedding of IL-2Rα. Shed receptor proteins may bind free IL-2, preventing its interaction with cells. However, the much greater affinity of IL-2R$\alpha\beta\gamma$ for IL-2 compared with IL-2Rα alone suggests that serum IL-2Rα is not likely to contribute significantly to immunosuppression. Clinically, an increased level of shed IL-2Rα in the serum is a marker of strong antigenic stimulation, e.g., acute rejection of a transplanted organ. Infection of T cells by human T lymphotrophic virus–1 (HTLV-1) activates IL-2Rα synthesis and also leads to shed IL-2Rα in the serum.

INTERLEUKIN-4

Interleukin-4 (IL-4) was initially identified as a helper T cell–derived cytokine of approximately 20 kD that stimulated the proliferation of mouse B cells in the presence of anti-Ig antibody (an analog of antigen) and

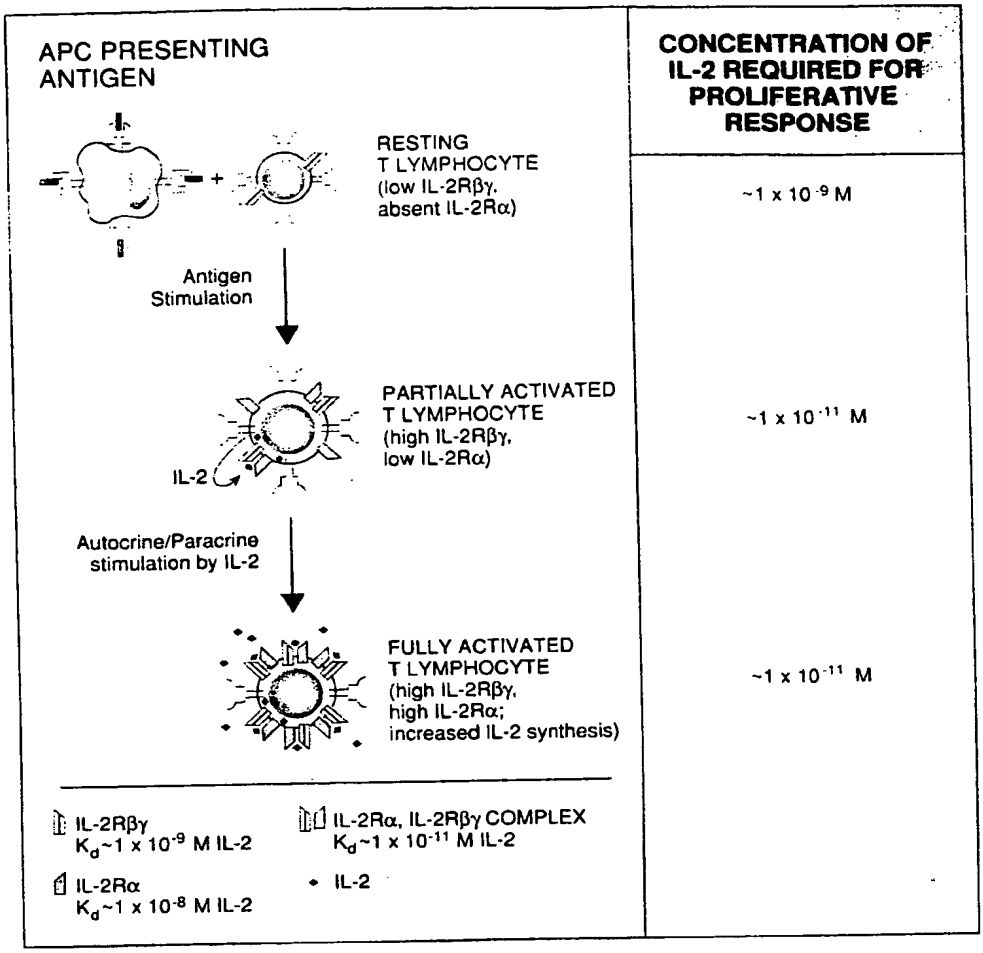


FIGURE 12–5. IL-2 receptors. *The high-affinity IL-2 receptor (IL-2R) is composed of a complex of two separate polypeptides (IL-2Rβγ and IL-2Rα) that interact to bind IL-2 with high affinity. Resting T cells express only IL-2Rβγ, which binds IL-2 with lower affinity. T cell activation by antigen and an antigen-presenting cell (APC) leads to IL-2Rα synthesis and expression, thereby increasing the affinity of the IL-2Rβγ receptor and allowing growth stimulation at physiologic IL-2 concentrations. IL-2 produced by the activated T cell, further increases IL-2Rα expression and stimulates IL-2 synthesis, providing a positive amplification system.*

caused enlargement of resting B cells as well as increased expression of class II MHC molecules. It is now known that the main physiologic function of IL-4 is as a regulator of allergic reactions (see Chapter 14). IL-4 is a member of the four α-helical cytokine family, and its receptor is a 130 kD protein that contains the conserved WSXWS motif (see Box 12–1). It is thought that IL-4 signaling involves clustering of the receptor by IL-4 homodimers, activating a receptor-associated tyrosine kinase. The principal cellular sources of IL-4 are $CD4^+$ T lymphocytes, specifically of the T_H2 subset (see Chapter 10). In fact, IL-4 production is used as the criterion for classifying $CD4^+$ T cells into this subset, with IFN-γ being the hallmark of the T_H1 cells. Activated mast cells and basophils, as well as some $CD8^-$ T cells, are also capable of producing IL-4.

IL-4 has important actions on several cell types.

1. *IL-4 is required for the production of IgE and is the principal cytokine that stimulates switching of B cells to this heavy chain isotype.* The mechanisms of this effect were described in Chapter 9. IgE is the principal mediator of immediate hypersensitivity (allergic) reactions, and enhanced production of IL-4 is believed to be central to the development of allergies (see Chapter 14). IgE antibodies also play a role in defense against helminthic infections, this being the principal known physiologic function of the T_H2 subset of helper T cells (see Chapter 16). Mice in which the IL-4 gene is disrupted fail to produce IgE. IL-4 also inhibits switching to IgG2a and IgG3 in mice, all of which are augmented by IFN-γ. This is one of several reciprocal antagonistic actions of IL-4 and IFN-γ.

2. IL-4 inhibits macrophage activation and blocks most of the macrophage activating effects of IFN-γ, including increased production of cytokines such as IL-1, nitric oxide, and prostaglandins. These effects are shared with those of IL-10, which is also produced by T_H2 cells. This is one of the main reasons why activation of T_H2 cells is often associated with a suppression of macrophage-mediated immune reactions (see Chapter 10).

3. IL-4 is a growth and differentiation factor for T cells, in particular for cells of the T_H2 subset. IL-4 promotes the development of IL-4 + IL-5–secreting T_H2 cells from naive T cells stimulated with antigen. Thus, stimuli that favor IL-4 production early after antigen exposure favor the development of T_H2 cells. This early source of IL-4 is not yet known. IL-4 also functions as the autocrine growth factor for differentiated T_H2 cells.

further promoting expansion of this subset. Mice lacking the IL-4 gene show a deficiency in the development and maintenance of T_H2 cells, even after stimuli (such as helminthic infections) that are normally potent inducers of this subset.

4. IL-4 stimulates the expression of certain adhesion molecules, notably vascular cell adhesion molecule–1 (VCAM-1), on endothelial cells, resulting in increased binding of lymphocytes, monocytes, and especially eosinophils. IL-4–treated endothelial cells also secrete the chemokine monocyte chemotactic protein–1 (MCP-1) and an as yet undefined chemokine that acts specifically on eosinophils. As a result, high local concentrations of IL-4 induce monocyte- and eosinophil-rich inflammatory reactions.

5. IL-4 is a growth factor for mast cells, and synergizes with interleukin-3 (IL-3) in stimulating mast cell proliferation.

Thus, IL-4 plays a critical role in IgE- and eosinophil-mediated inflammatory reactions. IL-4 antagonists are currently being tested in patients for controlling severe allergic reactions.

Another cytokine recently identified as a product of mouse T_H2 T cells is IL-13. Its biologic activities largely overlap those of IL-4 and will not be discussed separately.

TRANSFORMING GROWTH FACTOR–β

The original description of transforming growth factor–β was made in the field of tumor biology. It was noted that certain tumors produced activities, called transforming growth factor, that would allow normal cell types to grow in soft agar, a characteristic of malignant ("transformed") cells. Subsequently, it was found that growth stimulation was caused by one polypeptide, called TGF-α, but that survival in soft agar required a second factor, called TGF-β. TGF-α is a polypeptide growth factor for epithelial and mesenchymal cells and will not be discussed further. TGF-β is a family of closely related molecules, encoded by distinct genes, commonly designated TGF-β1, TGF-β2, and TGF-β3. (The TGF-β family also includes other members thought to be involved in normal development rather than immunity; these will not be discussed here.) Cells of the immune system (e.g., T cells and monocytes) synthesize mainly TGF-β1, but certain anatomic sites (e.g., within the central nervous system) may contain high levels of TGF-β3. Native TGF-β1 is a homodimeric protein of approximately 28 kD. TGF-β1 is synthesized in a latent form that must be activated by proteases. Both antigen-activated T cells and LPS-activated mononuclear phagocytes secrete biologically active TGF-β1. TGF-β receptors are not yet well defined, although one TGF-β–binding protein may be a serine/threonine kinase.

The actions of TGF-β are highly pleiotropic. TGF-β inhibits the growth of many cell types and stimulates the growth of others. Often, TGF-β can either inhibit or stimulate growth of the same cell type, depending upon culture conditions such as degree of confluence. TGF-β causes synthesis of extracellular matrix proteins, such as collagens, and of cellular receptors for matrix proteins. (The ability of TGF-β to induce extracellular matrix probably underlies its ability to promote cell growth in soft agar.) *In vivo*, TGF-β causes the growth of new blood vessels, a process called angiogenesis.

As a cytokine, TGF-β is potentially important because it antagonizes many responses of lymphocytes. For example, TGF-β inhibits T cell proliferation to polyclonal mitogens or in mixed leukocyte reactions (see Chapter 17) and inhibits maturation of CTLs. It can also inhibit macrophage activation. TGF-β also acts on other cells, such as polymorphonuclear leukocytes and endothelial cells, again largely to counteract the effects of pro-inflammatory cytokines. In this sense, TGF-β is an "anti-cytokine" and may be a signal for shutting off immune responses. Mice in which the TGF-β1 gene has been disrupted by knockout technology develop uncontrolled inflammatory reactions. Signals that cause T cells to synthesize TGF-β may cause them to behave as suppressor cells (see Chapter 10). *In vivo*, certain tumors may escape an immune response by secreting large quantities of TGF-β.

Although TGF-β is largely a negative regulator of immune responses, it may have some positive effects as well. For example, in mice, TGF-β has been shown to switch B cells to the IgA isotype, and it may therefore be important in the generation of mucosal immune responses that are mediated by IgA (discussed in Chapter 11).

Cytokines That Regulate Immune-Mediated Inflammation

We will now discuss a group of cytokines derived principally from antigen-activated $CD4^+$ and $CD8^+$ T lymphocytes that serve primarily to activate the functions of nonspecific effector cells. Thus, these cytokines play key roles in the effector phase of cell-mediated immune responses. The molecules described in this section are summarized in Table 12–3.

INTERFERON-γ

Interferon-γ (IFN-γ), also called immune or type II interferon, is a homodimeric glycoprotein containing approximately 21 to 24 kD subunits. The size variation of the subunit is caused by variable degrees of glycosylation, but each subunit contains an identical 18 kD polypeptide encoded by the same gene. IFN-γ is produced both by naive (T_H0) and T_H1 $CD4^-$ helper T cells and by nearly all $CD8^+$ T cells. Transcription is directly initiated as a consequence of antigen activation and is enhanced by IL-2 and IL-12. IFN-γ is also produced by natural killer (NK) cells, which are the principal source of this cytokine in T cell–deficient mice. (In this setting, IFN-γ may function as a mediator of natural immunity.)

As its name implies, IFN-γ shares many activities with type I IFN. Specifically, IFN-γ induces an antiviral state and is antiproliferative. However, IFN-γ binds to a unique cell surface receptor, different from but structurally related to that utilized by type I IFN. More importantly, IFN-γ has several properties related to im-

TABLE 12–3. Mediators of Immune-Mediated Inflammation

Cytokine	Number of Genes	Polypeptide Size	Cell Source	Cell Target	Primary Effects on Each Target
Gamma interferon	1	21–24 kD (homodimer)	T cell, NK cell	Mononuclear phagocyte	Activation
				Endothelial cell	Activation
				NK cell	Activation
				All	Increased class I and class II MHC molecules
Lymphotoxin	1	24 kD (homotrimer)	T cell	Neutrophil	Activation
				Endothelial cell	Activation
				NK cell	Activation
Interleukin-10	1	20 kD (homodimer)	T cell	Mononuclear phagocyte	Inhibition
				B cell	Activation
Interleukin-5	1	20 kD (homodimer)	T cell	Eosinophil	Activation
				B cell	Growth and activation
Interleukin-12	2	35–40 kD (heterodimer)	Macrophages	NK cells	Activation
				T cells	Activation (growth and differentiation)
Migration inhibition factor	?	?	T cell	Mononuclear phagocyte	Conversion from motile to immotile state

Abbreviations: NK, natural killer; kD, kilodalton; MHC, major histocompatibility complex.

munoregulation that separate it functionally from type I IFN.

1. *IFN-γ is a potent activator of mononuclear phagocytes.* It directly induces synthesis of the enzymes that mediate the respiratory burst, allowing macrophages to kill phagocytosed microbes. Along with second signals, such as LPS and perhaps TNF, it allows macrophages to kill tumor cells. Cytokines that cause such functional changes in mononuclear phagocytes have been called **macrophage-activating factors** (MAFs). *IFN-γ is the principal MAF and provides the means by which T cells activate macrophages.* Other MAFs include GM-CSF, and, to a lesser extent, IL-1 and TNF. Macrophage activation is described in more detail in Chapter 13. It is worth noting here that macrophage activation actually involves several different responses, and macrophages are said to be activated when they perform a particular function being assayed. For example, IFN-γ fully activates macrophages to kill phagocytosed microbes but only partly activates macrophages to kill tumor cells.

2. *IFN-γ increases class I MHC molecule expression and, in contrast to type I IFN, also causes a wide variety of cell types to express class II MHC molecules.* Thus, IFN-γ amplifies the cognitive phase of the immune response by promoting the activation of class II–restricted $CD4^+$ helper T cells (see Chapter 6, Fig. 6–5). *In vivo*, IFN-γ can enhance both cellular and humoral immune responses through these actions at the cognitive phase.

3. *IFN-γ acts directly on T and B lymphocytes to promote their differentiation.* IFN-γ promotes the differentiation of naive $CD4^+$ T cells to the T_H1 subset and inhibits the proliferation of T_H2 cells. IFN-γ is one of the cytokines required for the maturation of $CD8^+$ CTLs (see Chapter 13). It also acts on B cells to promote switching to the IgG2a and IgG3 subclasses in mice and to inhibit switching to IgG1 and IgE.

4. *IFN-γ activates neutrophils,* upregulating their respiratory burst. It is a less potent activator of neutrophils than TNF or lymphotoxin.

5. *IFN-γ stimulates the cytolytic activity of NK cells,* more so than type I IFN.

6. *IFN-γ is an activator of vascular endothelial cells,* promoting $CD4^+$ T lymphocyte adhesion and morphologic alterations that facilitate lymphocyte extravasation. As mentioned earlier, IFN-γ also potentiates many of the actions of TNF on endothelial cells.

The net effect of these varied activities of IFN-γ is to promote T_H1 and macrophage-rich inflammatory reactions, while suppressing T_H2 and eosinophil-rich reactions. Mice in which the IFN-γ or IFN-γ receptor genes have been disrupted show several immunologic defects, including increased susceptibility to infections with intracellular microbes (which cannot be cleared because of defective macrophage activation), reduced production of nitric oxide by macrophages from mice infected with mycobacteria, reduced serum levels of IgG2a and IgG3 antibodies, reduced expression of class II MHC molecules on macrophages from mycobacteria-infected mice, and defective NK cell function.

LYMPHOTOXIN

Lymphotoxin is a 21 to 24 kD glycoprotein that is approximately 30 per cent homologous to TNF and competes with TNF for binding to the same cell surface receptors. (As noted earlier, LT is sometimes called TNF-β.) In humans, LT and TNF genes are located in tandem within the MHC on chromosome 6 (see Chapter 5). LT is produced exclusively by activated T lymphocytes and is often produced coordinately with IFN-γ by such cells. Human LT, unlike TNF, contains one or two N-linked oligosaccharides (accounting for the variability in molecular sizes). In further contrast to TNF, LT is synthesized as a true secretory protein without a membrane-spanning region. A third member of the TNF/LT family has recently been described. The gene product,

tentatively named LT-β, is a cell surface protein that binds LT to form a cell surface complex that mediates the effects of LT on other cells.

Most studies have found little difference between the biologic effects of TNF and LT, consistent with their binding to the same receptor. The most important distinction between these cytokines appears to be that LT is synthesized exclusively by T cells, whereas TNF, although made by T cells, is predominantly derived from mononuclear phagocytes. In general, the quantities of LT synthesized by T cells are much less than the amounts of TNF made by LPS-stimulated mononuclear phagocytes, and LT is not readily detected in the circulation. Therefore, LT is usually a locally acting paracrine factor and not a mediator of systemic injury. Although neither TNF nor LT is toxic for normal (nonneoplastic) cells, both cytokines may contribute to CTL-mediated lysis of target cells (see Chapter 13). Like TNF, LT is a potent activator of neutrophils and thus provides lymphocytes with a means of regulating acute inflammatory reactions. It is more potent than IFN-γ as an activator of neutrophils, and the actions of LT are enhanced by IFN-γ. LT is also an activator of vascular endothelial cells, causing increased leukocyte adhesion, cytokine production, and morphologic changes that facilitate leukocyte extravasation. These effects, like those of TNF, are also enhanced by IFN-γ.

INTERLEUKIN-10

Interleukin-10 (IL-10) is an 18 kD cytokine produced by the T_H2 subset of $CD4^+$ helper cells. It is also produced by some activated B cells, by some T_H1 cells (in humans), by activated macrophages, and by some non-lymphocytic cell types (e.g., keratinocytes). IL-10 is a member of the four α-helical cytokine family and probably functions as a homodimer. The two major activities of IL-10 are to inhibit cytokine (i.e., TNF, IL-1, chemokine, and IL-12) production by macrophages, and to inhibit the accessory functions of macrophages in T cell activation. The latter effect is due to reduced expression of class II MHC molecules and reduced expression of certain costimulators (e.g., B7). The net effect of these actions is to inhibit T cell–mediated immune inflammation. In addition to its inhibitory effects on macrophages, IL-10 has stimulatory actions on B cells. It may be a switching factor for the production of IgG4 in humans (homologous to IgG1 in mice).

Studies of mice in which the IL-10 gene has been disrupted by knock-out technology reveal few immunologic abnormalities. Such mice develop intestinal inflammatory lesions, the basis of which is unexplained.

Interestingly, the genome of the Epstein-Barr virus contains a gene homologous to IL-10, and viral IL-10 shares *in vitro* activity with the T cell–derived cytokine. This raises the intriguing possibility that the virus has acquired the human gene as a means of inhibiting antiviral immunity.

INTERLEUKIN-5

Interleukin-5 (IL-5) is an approximately 40 kD homodimeric cytokine produced by the T_H2 subset of $CD4^+$ T cells and by activated mast cells. It belongs to the four α-helical cytokine family, although each bundle of four helices consists of three strands from one monomer and one strand from the other. The receptor contains the WSXWS motif and interacts with a 150 kD signal-transducing subunit shared with IL-3 and GM-CSF (see Box 12–1).

The major action of IL-5 is to stimulate the growth and differentiation of eosinophils and to activate mature eosinophils in such a way that they can kill helminths. In mice, neutralizing antibodies to IL-5 inhibit the eosinophilia seen in response to helminthic infection. This activity of IL-5 is complemented by the activities of IL-4 (e.g., IgE switching and eosinophil recruitment) and of IL-10 (e.g., IgG4 switching), contributing to T_H2-mediated allergic reactions (see Chapter 14). IL-5 also acts as a costimulator for the growth of antigen-activated mouse B cells and was previously called either B cell growth factor 2 or T cell replacing factor. IL-5 may function synergistically with other cytokines, such as IL-2 and IL-4, to stimulate the growth and differentiation of B cells. IL-5 has also been found to act on more mature B cells to cause increased synthesis of immunoglobulin, especially of IgA. These actions are discussed in greater detail in Chapter 9.

INTERLEUKIN-12

Interleukin-12 is a 70 kD heterodimer consisting of two covalently linked polypeptide chains, one of 35 kD (p35) and the other of 40 kD (p40). The p35 subunit is produced by many cell types, including T and B lymphocytes, NK cells, and monocytes. The p40 chain is produced mainly by activated monocytes and B cells, so that these are the principal sources of the complete cytokine. (In this functional category of cytokines, IL-12 is the only one that is not produced by T cells, and because of its action on NK cells, can also be considered a mediator of natural immunity.) The p35 protein has a four α-helix structure, similar to that of many other cytokines. Interestingly, the p40 component of IL-12 is homologous to the IL-6 receptor, containing an Ig domain and a WSXWS motif. Thus, the intact heterodimer appears to be composed of one cytokine-like protein and one cytokine receptor–like protein. Binding studies indicate that the true receptor for IL-12 is expressed on activated T and NK cells, but this receptor has not yet been fully characterized.

IL-12 is an important regulator of cell-mediated immune responses because of its effects on NK cells and T lymphocytes.

1. *IL-12 is the most potent NK cell stimulator known.* It induces transcription of IFN-γ by NK cells, and shows a strong synergy with IL-2. In addition to stimulating IFN-γ production, IL-12 enhances the cytolytic activity of NK cells and is a growth factor for these cells.

2. *IL-12 stimulates the differentiation of naive $CD4^+$ T cells to the T_H1 subset.* The development of a T_H1 versus T_H2 dominant T cell response may be controlled by the relative production of IL-12 and IFN-γ, which

favor T_H1 differentiation, and IL-4 and IL-10, which promote T_H2 differentiation (see Chapter 10).

3. *IL-12 stimulates the differentiation of $CD8^+$ T cells into mature, functionally active CTLs.* Because of this effect, IL-12 has some potential in the treatment of disseminated cancers.

Thus, IL-12 is an important regulator of the effector phase of cell-mediated immune reactions. It serves this function by directly activating some effector cells, and by regulating the development of other effector cells.

MIGRATION INHIBITION FACTOR

We conclude our discussion of cytokines that regulate effector cells by considering the issue of migration inhibition factor (MIF). One early view of cell-mediated immune reactions proposed that mononuclear phagocyte accumulation in tissues depended on the retention of such cells in response to locally produced cytokines that inhibit motility. It now seems more likely that retention of leukocytes in the tissues is controlled primarily by expression of specific receptors for extracellular matrix molecules, such as integrins (see Box 7–3, Chapter 7), and CD44. Nevertheless, one of the first cytokine activities identified was one that inhibited macrophage motility *in vitro*, called migration inhibition factor. MIF has still not been identified as a unique cytokine. At present, both the biochemical identity and biologic significance of MIF remain unclear.

Cytokines That Stimulate Hematopoiesis

Several of the cytokines generated during both natural immunity and antigen-induced specific immune responses have potent stimulatory effects on the growth and differentiation of bone marrow progenitor cells. Thus, immune and inflammatory reactions, which consume leukocytes, also elicit production of new leukocytes to replace inflammatory cells. All of the various mature leukocyte cell populations arise as a consequence of progressive expansion and irreversible differentiation of the progeny of self-renewing pluripotent stem cells. Maturation of hematopoietic cells involves commitment to a particular lineage and occurs concomitantly with loss of ability to develop into other mature cell types. This process has been depicted as a simple tree. The cytokines that stimulate expansion and differentiation of bone marrow progenitor cells are collectively called **colony-stimulating factors** (CSFs) because they are often assayed by their ability to stimulate the formation of cell colonies in bone marrow cultures. These colonies of cells mature during the *in vitro* assay, acquiring characteristics of specific cell lineages (e.g., granulocytes, mononuclear phagocytes). Different CSFs act on bone marrow cells at different stages of maturation and preferentially promote development of colonies of different lineages (Fig. 12–6). The names assigned to CSFs reflect the types of colonies that arise in these assays. Interestingly, many of the CSFs are located in a gene cluster on human chromosome 5, including IL-3 and GM-CSF. IL-4 and IL-5 have been mapped to this same complex.

Some of the actions of CSFs are influenced by other cytokines. For example, TNF, LT, IFN-γ, and TGF-β all inhibit growth of bone marrow progenitor cells. In contrast, IL-1 and IL-6 enhance responses to CSFs. In general, cytokines are thought both to be necessary for normal marrow function and to provide a means of fine tuning function in response to stimulation. Some of the specific CSFs are listed in Table 12–4.

c-KIT LIGAND

The pluripotent stem cell expresses a tyrosine-kinase membrane receptor that has been identified as the protein product of the cellular oncogene, *c-kit*. The extracellular portion of this receptor contains five Ig domains. The cytokine that interacts with the receptor has been called **c-kit ligand**, and is also referred to as "stem cell factor." c-Kit ligand is synthesized by stromal cells of the bone marrow (including adipocytes, fibroblasts, and endothelial cells) in two forms: a transmembrane protein of about 27 kD and a secreted form of about 24 kD. These different products result from alternative splicing of the same gene. The soluble form of this ligand is absent in a mutant mouse strain called steel, and the soluble form of the c-kit ligand is thus sometimes called *steel factor*. The steel mouse has only selective gaps in its bone marrow–derived cell populations (e.g., inadequate mast cell and eosinophil production), which has led to the conclusion that the cell surface form of c-kit ligand is more important than the soluble form for stimulating stem cells to mature into various hematopoietic lineages. Elimination of both forms of c-kit ligand, by complete knockout of the gene, is lethal.

It is not yet possible to purify large numbers of stem cells for direct analysis. Many of the conclusions about c-kit ligand and other early acting CSFs are derived from experiments in which populations enriched from stem cells are exposed to the cytokines in culture, and the types of colonies that develop are analyzed. From this kind of experiment, it is believed that c-kit ligand is needed to make stem cells responsive to other CSFs, but that it does not cause colony formation by itself. Bone marrow cell cultures that contain stromal cells do not have a requirement for exogenous c-kit ligand since the stromal cells express this gene product.

INTERLEUKIN-3

Interleukin-3 (IL-3), also known as **multilineage colony-stimulating factor** (multi-CSF), is a 20 to 26 kD product of $CD4^+$ T cells that acts on the most immature marrow progenitors and promotes the expansion of cells that differentiate into all known mature cell types. IL-3 is a member of the four α-helix family of cytokines. In humans, the receptor consists of a unique WSXWS-containing subunit and a 150 kD signal-transducing

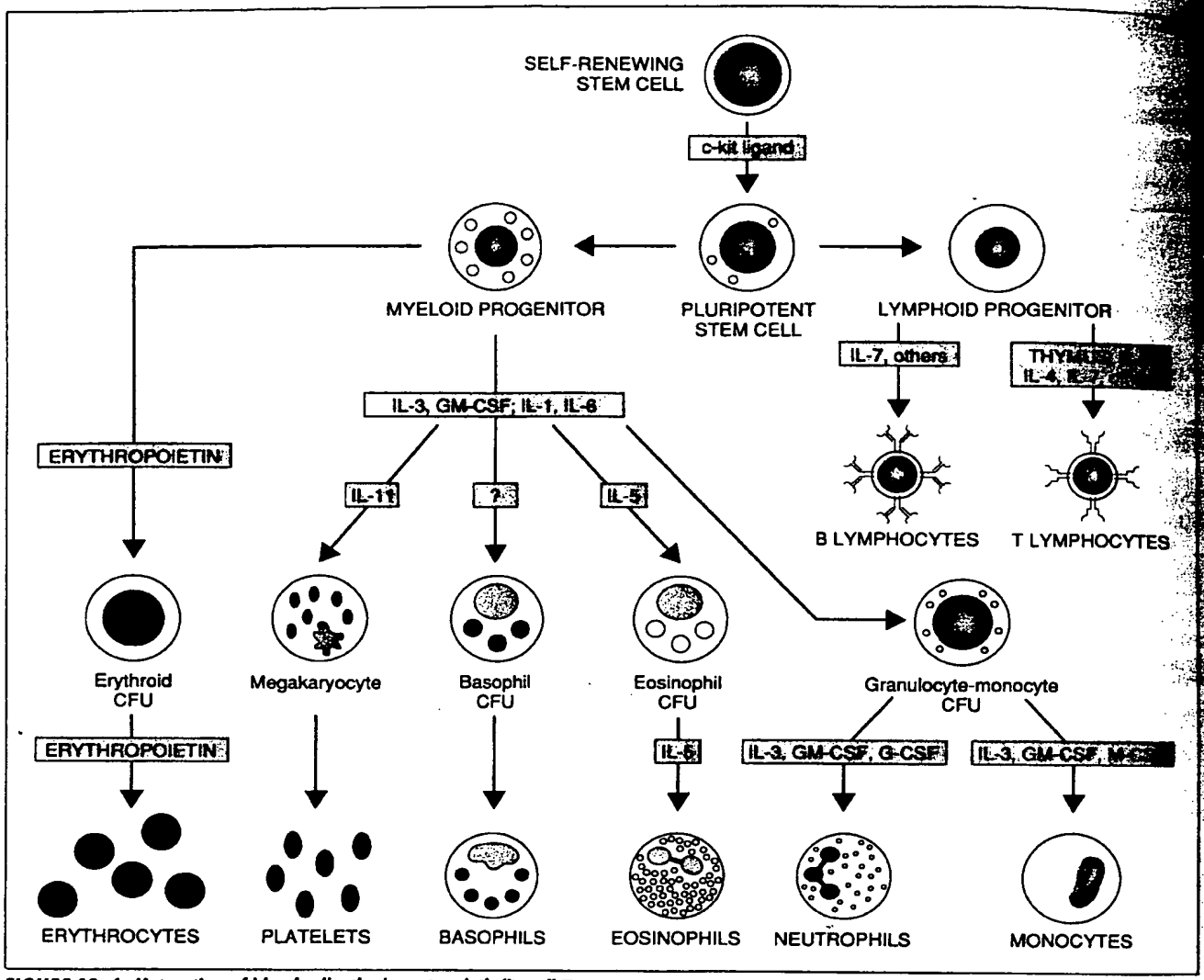


FIGURE 12–6. Maturation of blood cells: the hematopoietic "tree." *The maturation of different lineages of blood cells is regulated by various cytokines. CFU, colony-forming unit; IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor.*

subunit shared with IL-5 and GM-CSF. In the mouse, the signal-transducing subunit is unique. Most functional analyses of IL-3 have been performed in mice. It has been found that IL-3 also promotes the growth and development of mast cells from bone marrow–derived progenitors, an action enhanced by IL-4. IL-3 is produced by $CD4^+$ helper T cells of both the T_H1 and T_H2 subsets. Human IL-3 has been identified by the complementary DNA (cDNA) cloning of a molecule homologous to mouse IL-3. Although IL-3 is made by some human T cell clones, it has been harder to establish a role for this cytokine in experimental systems of hematopoiesis in humans. In fact, many actions attributed to murine IL-3 appear to be performed by human granulocyte-macrophage CSF (see below). It is not known whether these experimental results reflect differences in species or in experimental conditions. If these are a species difference, they may be related to the differences in IL-3 receptor structure described above.

GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a 22 kD glycoprotein made by activated T cells and by activated mononuclear phagocytes, vascular endothelial cells, and fibroblasts. GM-CSF is a member of the four α-helix family of cytokines. Its receptor consists of a unique WSXWS-containing subunit and a 150 kD signal-transducing subunit. As noted above, this latter subunit is shared with the IL-5 receptor and, in humans but not mice, with the IL-3 receptor. GM-CSF in the mouse acts primarily on bone marrow progenitors already committed to develop into leuko-

TABLE 12-4. Mediators of Immature Leukocyte Growth and Differentiation

Cytokine	Number of Genes	Polypeptide Size	Cell Source	Cell Target	Primary Effects on Each Target
c-Kit ligand	1	24 kD (monomer)	Bone marrow stromal cell	Pluripotent stem cell	Activation
Interleukin-3	1	20–26 kD (dimer)	T cell	Immature progenitor	Growth and differentiation to all cell lines
Granulocyte-macrophage CSF	1	22 kD (dimer)	T cell, mononuclear phagocyte, endothelial cell, fibroblast	Immature progenitor Committed progenitor Mononuclear phagocyte	Growth and differentiation to all cell lines Differentiation to granulocytes and mononuclear phagocytes Activation
Macrophage CSF	1	40 kD (dimer)	Mononuclear phagocyte, endothelial cell, fibroblast	Committed progenitor	Differentiation to mononuclear phagocytes
Granulocyte CSF	1	19 kD (dimer)	Mononuclear phagocyte, endothelial cell, fibroblast	Committed progenitor	Differentiation to granulocytes
Interleukin-7	1	25 kD (monomer)	Fibroblast, bone marrow stromal cells	Immature progenitor	Growth and differentiation to B lymphocytes

Abbreviations: CSF, colony-stimulating factor; kD, kilodalton.

cytes and thus presumably acts on a more differentiated population than IL-3. In human systems, however, GM-CSF also promotes growth of cells not yet committed to form leukocytes (e.g., platelets and progenitors of red blood cells), replacing IL-3. GM-CSF also activates mature leukocytes. For example, it mimics some of the actions of IFN-γ as an activator of macrophages, although it is less potent.

GM-CSF is not detected in the circulation and presumably acts locally at sites of production. Thus, in peripheral tissues T cell– and macrophage-derived GM-CSF may function mainly to activate mature leukocytes at sites of immune inflammatory responses, whereas hematopoietic effects may be mediated by GM-CSF produced by T cells, endothelial cells, or stromal fibroblasts in the bone marrow. Recombinant GM-CSF has been used to stimulate the bone marrow of patients with defects in hematopoiesis and to stimulate bone marrow recovery after cytotoxic chemotherapy or bone marrow transplantation.

MONOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR

Monocyte-macrophage colony-stimulating factor (M-CSF), also called CSF-1, is made by macrophages and by endothelial cells and fibroblasts. The secreted polypeptide is approximately 40 kD and forms a stable dimer. The M-CSF receptor is structurally related to c-kit. It contains five extracellular Ig domains and an intracellular tyrosine kinase. The M-CSF receptor gene was initially identified as the normal cellular counterpart of a viral oncogene, *v-fms*.

M-CSF acts primarily on those progenitors that are already committed to develop into monocytes and are presumably more mature than the targets for GM-CSF. Like GM-CSF, M-CSF does not circulate, and the major colony-stimulating effect may be derived from local production within the marrow cavity.

GRANULOCYTE COLONY-STIMULATING FACTOR

Granulocyte colony-stimulating factor (G-CSF) is made by the same cells that make GM-CSF. The secreted polypeptide is approximately 19 kD and probably forms a dimer. G-CSF is a member of the four α-helical cytokine family, and its receptor contains the WSXWS motif. In contrast to other CSFs, G-CSF does normally circulate. It acts primarily on marrow progenitors already committed to develop into granulocytes, again a more mature population than that responsive to GM-CSF. Because G-CSF can act at a distance, neutrophil maturation and release from bone marrow are highly influenced by inflammatory reactions occuring in the periphery, outside the marrow.

INTERLEUKIN-7

Interleukin 7 (IL-7) is a cytokine secreted by marrow stromal cells that acts on hematopoietic progenitors committed to the B lymphocyte lineage. Most models of hematopoiesis suggest that lymphocyte progenitors differentiate from common stem cells very early in maturation, so that IL-7 is probably acting on cells at the same level of development as IL-3 or GM-

CSF. Recent studies suggest that IL-7 may also stimulate the growth and maturation of immature $CD4^-CD8^-$ T cell precursors in the thymus. However, this is based on *in vitro* experiments, and the cellular source of IL-7 in the thymus is not known. Transgenic mice that overexpress IL-7 show markedly increased numbers of pre–B cells in the bone marrow and peripheral lymphoid tissues.

OTHER COLONY-STIMULATING CYTOKINES

IL-9 is a 30 to 40 kD protein that supports the growth of some T cell lines and of bone marrow–derived mast cell progenitors. It may also stimulate development of other lineages from marrow-derived precursors. However, it is not known whether IL-9 has an effect on normal lymphocytes (other than cell lines) or if it plays a role in the regulation of immune responses or hematopoiesis *in vivo*.

IL-11 is a ~20 kD cytokine produced by bone marrow stromal cells, especially after activation (which may be achieved experimentally by pharmacologic agents such as phorbol esters). IL-11 stimulates megakaryopoiesis and may prove to be of therapeutic benefit in patients with platelet deficiencies. It also enhances the development of macrophages and perhaps other cell lineages from marrow precursors.

SUMMARY

Cytokines are a family of protein mediators of both natural and acquired immunity. The same cytokines are often made by many cell types, and individual cytokines often act on many cell types. The actions of different cytokines are often redundant and influence the action of other cytokines. In general, cytokines are synthesized in response to inflammatory or antigenic stimuli and act locally, in an autocrine or paracrine fashion, by binding to high affinity receptors on target cells. Certain cytokines may be produced in sufficient quantity to circulate and exert endocrine actions. For many cell types, cytokines serve as growth factors.

We have classified cytokines into four groups, according to their principal actions:

The first group consists of those cytokines that mediate natural immunity and includes the antiviral type I interferons and the pro-inflammatory cytokines—tumor necrosis factor, interleukin-1, interleukin-6, and members of the newly described family of chemokines. The predominant cellular source of these molecules is mononuclear phagocytes.

The second group of cytokines is derived largely from antigen-stimulated $CD4^+$ T lymphocytes and serves to regulate the activation, growth, and differentiation of B and T cells. This group includes interleukin-2, the principal T cell growth factor; interleukin-4, the major regulator of IgE synthesis; and transforming growth factor–β, which inhibits lymphocyte responses.

The third group of cytokines, produced by antigen-activated $CD4^+$ and $CD8^+$ T lymphocytes, serves to activate inflammatory leukocytes and places these effector cells under T cell regulation. This group includes interferon-γ, the principal activator of mononuclear phagocytes; lymphotoxin, an activator of neutrophils; interleukin-10, a negative regulator of mononuclear phagocyte function; interleukin-5, an activator of eosinophils; and interleukin-12 (produced by mononuclear phagocytes), a stimulator of NK cells and T cells.

The fourth group, collectively called colony-stimulating factors, consists of cytokines derived from marrow stromal cells and T cells, which stimulate the growth of bone marrow progenitors, thereby providing a source of additional inflammatory leukocytes.

Thus, cytokines serve many functions that are critical to host defense against pathogens and provide links between specific and natural immunity. Cytokines also regulate the magnitude and nature of immune responses by influencing the growth and differentiation of lymphocytes. Finally, cytokines provide important amplification mechanisms that enable small numbers of lymphocytes specific for any one antigen to activate a variety of effector mechanisms to eliminate the antigen. Excessive production or actions of cytokines can lead to tissue injury and even death. The administration of cytokines or their inhibitors is a potential approach for modifying biologic responses associated with disease.

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